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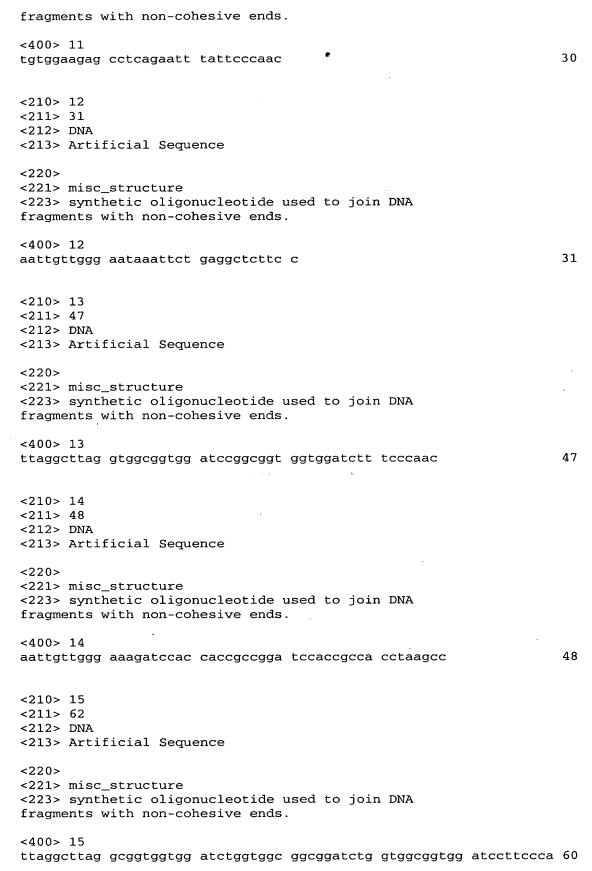
<110> Rosen, Craig A.
      Haseltine, William A.

<120> Albumin Fusion Proteins

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with non-cohesive ends.

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gataaagatt cccaac                                                    16


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with non-cohesive ends.

<400> 4
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<223> synthetic oligonucleotide used to join DNA fragments
with non-cohesive ends.

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ttaggcttat tcccaac                                                   17


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<213> Artificial Sequence

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with non-cohesive ends.

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aattgttggg aataagcc                                                  18


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<220>
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<222> 20)..(24)
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2

<223> first 5 amino acids of mature human serum albumin

<400> 7
Met Leu Leu Gln Ala Phe Leu Phe Leu Leu Ala Gly Phe Ala Ala Lys
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Ile Ser Ala Asp Ala His Lys Ser
              20

<210> 8
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fragments with non-cohesive ends.

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fragments with non-cohesive ends.

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ctcttgtgtg catcgaagcc acag   24

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3

fragments with non-cohesive ends.

<400> 11
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<210> 12
<211> 31
<212> DNA
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<220>
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fragments with non-cohesive ends.

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<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 13
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<210> 14
<211> 48
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 14
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<210> 15
<211> 62
<212> DNA
<213> Artificial Sequence

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<221> misc_structure
<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

<400> 15
ttaggcttag gcggtggtgg atctggtggc ggcggatctg gtggcggtgg atccttccca 60

<210> 16
<211> 63
<212> DNA
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<223> synthetic oligonucleotide used to join DNA
fragments with non-cohesive ends.

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gcc   63

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<400> 17
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 1               5                  10                  15

gaa aat ttc aaa gcc ttg gtg ttg att gcc ttt gct cag tat ctt cag    96
Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
                20                  25                  30

cag tgt cca ttt gaa gat cat gta aaa tta gtg aat gaa gta act gaa   144
Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
            35                  40                  45

ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa   192
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
        50                  55                  60

tca ctt cat acc ctt ttt gga gac aaa tta tgc aca gtt gca act ctt   240
Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65                  70                  75                  80

cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct   288
Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
                85                  90                  95

gag aga aat gaa tgc ttc ttg caa cac aaa gat gac aac cca aac ctc   336
Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
            100                 105                 110

ccc cga ttg gtg aga cca gag gtt gat gtg atg tgc act gct ttt cat   384
Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
            115                 120                 125
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5

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gac aat gaa gag aca ttt ttg aaa aaa tac tta tat gaa att gcc aga      432
Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
    130             135             140

aga cat cct tac ttt tat gcc ccg gaa ctc ctt ttc ttt gct aaa agg      480
Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145             150             155             160

tat aaa gct gct ttt aca gaa tgt tgc caa gct gct gat aaa gct gcc      528
Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
                165             170             175

tgc ctg ttg cca aag ctc gat gaa ctt cgg gat gaa ggg aag gct tcg      576
Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
            180             185             190

tct gcc aaa cag aga ctc aaa tgt gcc agt ctc caa aaa ttt gga gaa      624
Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
            195             200             205

aga gct ttc aaa gca tgg gca gtg gct cgc ctg agc cag aga ttt ccc      672
Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
        210             215             220

aaa gct gag ttt gca gaa gtt tcc aag tta gtg aca gat ctt acc aaa      720
Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225             230             235             240

gtc cac acg gaa tgc tgc cat gga gat ctg ctt gaa tgt gct gat gac      768
Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
                245             250             255

agg gcg gac ctt gcc aag tat atc tgt gaa aat cag gat tcg atc tcc      816
Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
            260             265             270

agt aaa ctg aag gaa tgc tgt gaa aaa cct ctg ttg gaa aaa tcc cac      864
Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
            275             280             285

tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca      912
Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
        290             295             300

tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct      960
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305             310             315             320

gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga     1008
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
                325             330             335

agg cat cct gat tac tct gtc gtg ctg ctg ctg aga ctt gcc aag aca     1056
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
                340             345             350

tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa     1104
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
            355             360             365
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6

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tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct    1152
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370             375             380

cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag    1200
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385             390             395             400

tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc    1248
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
                405             410             415

caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa    1296
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420             425             430

gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt    1344
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
            435             440             445

gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat    1392
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450             455             460

gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc    1440
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465             470             475             480

ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca    1488
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
                485             490             495

tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat    1536
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500             505             510

ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca    1584
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
            515             520             525

ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg    1632
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530             535             540

aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag    1680
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545             550             555             560

gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt    1728
Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
            565             570             575

gct gca agt caa gct gcc tta ggc tta taacatctac atttaaaagc atctcag 1782
Ala Ala Ser Gln Ala Ala Leu Gly Leu
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7

<212> PRT
<213> Homo Sapiens

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Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
              20   25   30

Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
          35   40   45

Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
      50   55   60

Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65   70   75   80

Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
              85   90   95

Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
              100   105   110

Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His
          115   120   125

Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg
      130   135   140

Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg
145   150   155   160

Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala
              165   170   175

Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser
          180   185   190

Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu
          195   200   205

Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro
      210   215   220

Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys
225   230   235   240

Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp
              245   250   255

Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser
          260   265   270

Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His
          275   280   285

Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser
          290   295   300

8

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Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala
305             310             315                 320

Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg
            325             330             335

Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr
            340             345             350

Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu
        355             360             365

Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro
    370             375             380

Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu
385             390             395                 400

Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro
            405             410             415

Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys
            420             425             430

Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys
            435             440             445

Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His
    450             455             460

Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser
465             470             475                 480

Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr
            485             490             495

Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp
            500             505             510

Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala
    515             520             525

Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu
    530             535             540

Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys
545             550             555                 560

Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val
            565             570             575

Ala Ala Ser Gln Ala Ala Leu Gly Leu
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<210> 20
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<400> 20
aatcgatgag caacctcact cttgtgtgca tctcttttct cgaggctcct ggaataag   58

<210> 21
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site in pPPC0006

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tacaaactta agagtccaat tagc   24

<210> 22
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site in pPPC0006

<400> 22
cacttctcta gagtggtttc atatgtctt   29

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<223> Synthetic oligonucleotide used to alter restriction
sites in pPPC0007

<400> 23
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<210> 24
<211> 60
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sites in pPPC0007

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<211> 32
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<223> forward primer useful for generation of albumin
fusion protein in which the albumin moiety is N-terminal
of the Therapeutic Protein

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11

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<210> 26
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<213> Artificial Sequence

<220>
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<223> reverse primer useful for generation of albumin
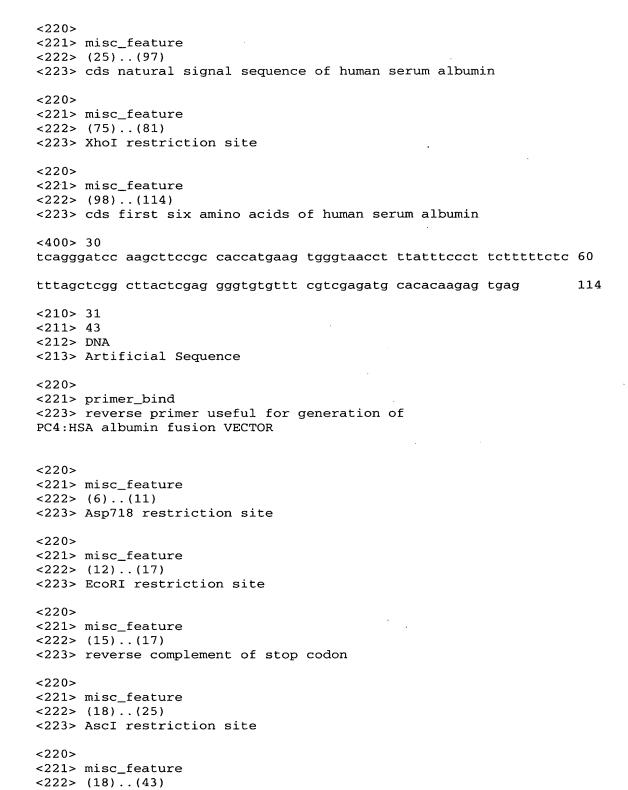fusion protein in which the albumin moiety is N-terminal
of the Therapeutic Protein

12

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protein in which the albumin  moiety is c-terminal of the
Therapeutic Protein

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fusion protein in which the albumin  moiety is c-terminal of
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<220>
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<220>
<221> misc feature
<222> (49)
<223> n equals a,t,g, or c
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<220>
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<400> 28
ctttaaatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnn nn          52


<210> 29
<211> 24
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> signal peptide of natural human serum albumin protein

<400> 29
Met Lys Trp Val Ser Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala
 1               5                   10                  15

Tyr Ser Arg Ser Leu Asp Lys Arg
            20

<210> 30
<211> 114
<212> DNA
<213> Artificial Sequence

<220>
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<223> forward primer useful for generation of PC4:HSA
albumin fusion VECTOR

<220>
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<222> (5)..(10)
<223> BamHI retsriction site

<220>
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<222> (11)..(16)
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<220>
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<222> (17)..(27)
<223> Kozak sequence
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<220>
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<222> (25)..(97)
<223> cds natural signal sequence of human serum albumin

<220>
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<222> (75)..(81)
<223> XhoI restriction site

<220>
<221> misc_feature
<222> (98)..(114)
<223> cds first six amino acids of human serum albumin

<400> 30
tcagggatcc aagcttccgc caccatgaag tgggtaacct ttatttccct tctttttctc 60

tttagctcgg cttactcgag gggtgtgttt cgtcgagatg cacacaagag tgag   114

<210> 31
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<221> primer_bind
<223> reverse primer useful for generation of
PC4:HSA albumin fusion VECTOR

<220>
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<222> (6)..(11)
<223> Asp718 restriction site

<220>
<221> misc_feature
<222> (12)..(17)
<223> EcoRI restriction site

<220>
<221> misc_feature
<222> (15)..(17)
<223> reverse complement of stop codon

<220>
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<222> (18)..(25)
<223> AscI restriction site

<220>
<221> misc_feature
<222> (18)..(43)
<223> reverse complement of DNA sequence encoding last 9 amino acids

<400> 31
gcagcggtac cgaattcggc gcgccttata agcctaaggc agc   43

<210> 32

18

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<211> 46
<212> DNA
<213> Artificial Sequence

<220>
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<223> forward primer useful for inserting Therapeutic
protein into pC4:HSA vector

<220>
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<220>
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<220>
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<222> (45)
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<220>
<221> misc feature
<222> (46)
<223> n equals a,t,g, or c


<400> 32
ccgccgctcg aggggtgtgt ttcgtcgann nnnnnnnnnn nnnnnn                46


<210> 33
<211> 55
<212> DNA
<213> Artificial Sequence


<220>
<221> primer_bind
<223> reverse primer useful for inserting Therapeutic
protein into pC4:HSA vector


<220>
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<222> (38)
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<220>
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<222> (39)
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<223> n equals a,t,g, or c

<220>
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<220>
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<220>
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<223> n equals a,t,g, or c

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agtcccatcg atgagcaacc tcactcttgt gtgcatcnnn nnnnnnnnn nnnnn   55

<210> 34
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Stanniocalcin signal peptide

<400> 34
Met Leu Gln Asn Ser Ala Val Leu Leu Leu Leu Val Ile Ser Ala Ser
 1   5   10   15

Ala

<210> 35
<211> 22
<212> PRT
<213> Artificial Sequence

<220>
<221> signal
<223> Synthetic signal peptide

<400> 35
Met Pro Thr Trp Ala Trp Trp Leu Phe Leu Val Leu Leu Leu Ala Leu
 1   5   10   15

Trp Ala Pro Ala Arg Gly
            20

22